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Published in:
International Journal of Developmental Neuroscience

DOI:
[10.1016/j.ijdevneu.2018.11.007](https://doi.org/10.1016/j.ijdevneu.2018.11.007)

Publication date:
2019

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Document Version
Peer reviewed version

[Link to publication in Discovery Research Portal](#)

Citation for published version (APA):

Andaloussi, Z. I. L., Taghzouti, K., & Abboussi, O. (2019). Behavioural and epigenetic effects of paternal exposure to cannabinoids during adolescence on offspring vulnerability to stress. *International Journal of Developmental Neuroscience*, 72, 48-54. <https://doi.org/10.1016/j.ijdevneu.2018.11.007>

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Article title: Behavioural and epigenetic effects of paternal exposure to cannabinoids during adolescence on offspring vulnerability to stress

Article reference: DN2320

Journal title: International Journal of Developmental Neuroscience

Corresponding author: Dr Oualid Abboussi

First author: Ms Zineb Ibn Lahmar Andaloussi

First published version available online: 23-NOV-2018

DOI information: 10.1016/j.ijdevneu.2018.11.007

{ HYPERLINK "<https://doi.org/10.1016/j.ijdevneu.2018.11.007>" \t "_blank" }

Accepted Manuscript version of Andaloussi, ZIL, Taghzouti, K & Abboussi, O 2019, 'Behavioural and epigenetic effects of paternal exposure to cannabinoids during adolescence on offspring vulnerability to stress', International Journal of Developmental Neuroscience, vol. 72, pp. 48-54. <https://doi.org/10.1016/j.ijdevneu.2018.11.007>

Behavioural and epigenetic effects of paternal exposure to cannabinoids during adolescence on offspring vulnerability to stress

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Abstract

Chronic cannabinoid exposure during adolescence in male rats induces chronic cognitive and emotional impairments. However, the impact of this form of exposure on offspring vulnerability to stress is unknown.

The aim of this study was to evaluate the behavioural and epigenetic effects of stress in the offspring of male rats whose fathers were exposed to cannabinoids during adolescence. Male adolescent offspring of Win55,212-2 (1.2 mg/kg) treated rats were exposed during one week to variable stressors and subjected to behavioural tests of anxiety and episodic-like memory, followed by an assessment of global DNA methylation and expression of DNA methyltransferases enzymes DNMT1 and DNMT3a mRNA in the prefrontal cortex.

Stress exposure during adolescence induced a significant anxiogenic-like effect but did not affect the episodic-like memory in the offspring of Win55,212-2 exposed fathers in comparison to the offspring of non-exposed fathers. These behavioural changes were subsequent to a significant increase in global DNA methylation and DNMT1 and DNMTa3 transcription in the prefrontal cortex.

These data suggest that the deleterious effect of chronic exposure to cannabinoids during adolescence are not limited to the exposed individuals but may increase the vulnerability to stress-induced anxiety in the offspring and alter their epigenetic programming.

Keywords: cannabinoids; adolescence; stress, offspring; DNA methylation; anxiety.

1. Introduction

Cannabis is one of the most widely used illicit substances in the world { ADDIN EN.CITE <EndNote><Cite><Author>Miech</Author><Year>2017</Year><RecNum>1554</RecNum><DisplayText>(1)</DisplayText><record><rec-number>1554</rec-number><foreign-keys><key app="EN" db-id="50wxdpzd9vd5r7e9t5b595djrfptrxw9avp">1554</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Miech, Richard</author><author>Johnston, Lloyd</author><author>O'Malley, Patrick M</author></authors></contributors><titles><title>Prevalence and attitudes regarding marijuana use among adolescents over the past decade</title><secondary-title>Pediatrics</secondary-title></titles><periodical><full-title>Pediatrics</full-title><abbr-1>Pediatrics</abbr-1><abbr-2>Pediatrics</abbr-2></periodical><pages>e20170982</pages><dates><year>2017</year></dates><isbn>0031-4005</isbn><urls></urls></record></Cite></EndNote>}. It presents a subject of intense political and social debates across the world concerning its legalization for medicinal and/or recreational use { ADDIN EN.CITE <EndNote><Cite><Author>Hughes</Author><Year>2018</Year><RecNum>1561</RecNum><DisplayText>(2, 3)</DisplayText><record><rec-number>1561</rec-number><foreign-keys><key app="EN" db-id="50wxdpzd9vd5r7e9t5b595djrfptrxw9avp">1561</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Hughes, Brendan</author><author>Wiessing, Lucas</author><author>Des Jarlais, Don</author><author>Griffiths, Paul</author></authors></contributors><titles><title>Could cannabis liberalisation lead to wider changes in drug policies and outcomes?</title><secondary-title>International Journal of Drug Policy</secondary-title></titles><periodical><full-title>International Journal of Drug Policy</full-title></periodical><pages>156-159</pages><volume>51</volume><dates><year>2018</year></dates><isbn>0955-3959</isbn><urls></urls></record></Cite><Cite><Author>Crume</Author><Year>2018</Year><RecNum>1562</RecNum><record><rec-number>1562</rec-number><foreign-keys><key app="EN" db-id="50wxdpzd9vd5r7e9t5b595djrfptrxw9avp">1562</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Crume, Tessa

L Juhl, Ashley Brooks-Russell, Ashley Hall, Katelyn E Wymore, Erica Borgelt, Laura M

Cannabis use during the perinatal period in a state with legalized recreational and medical marijuana: The association between maternal characteristics, breastfeeding patterns, and neonatal outcomes

The Journal of pediatrics

90-96

197

2018

0022-3476

}. Thus, there has been a surge of public and research interest in physiological and behavioural consequences of prenatal and postnatal cannabis exposure { ADDIN EN.CITE { ADDIN EN.CITE.DATA }}. Surprisingly, the implication of paternal cannabis use in the development of psychoactive disorders in the offspring has received little attention.

The cannabinoids such as the tetrahydrocannabinol-9 (THC), the psychoactive compound found in the cannabis plant, and the synthetic cannabinoid receptor agonist WIN55,212-2 (WIN) modulate the activity of the endocannabinoid system which plays a major role in coping and adaptation to stress through its interaction with the endocrine system { ADDIN EN.CITE <EndNote><Cite><Author>Morena</Author><Year>2016</Year><RecNum>1549</RecNum><DisplayText>(5)</DisplayText><record><rec-number>1549</rec-number><foreign-keys><key app="EN" db-id="50wxdpzd9vd5r7e9t5b595djrfptrxw9avp">1549</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Morena, Maria</author><author>Patel, Sachin</author><author>Bains, Jaideep S</author><author>Hill, Matthew N</author></authors></contributors><titles><title>Neurobiological interactions between stress and the endocannabinoid system</title><secondary-title>Neuropsychopharmacology</secondary-title></titles><periodical><full-title>Neuropsychopharmacology</full-title><abbr-1>Neuropsychopharmacology</abbr-1><abbr-2>Neuropsychopharmacology</abbr-2></periodical><pages>80</pages><volume>41</volume><number>1</number><dates><year>2016</year></dates><isbn>1740-634X</isbn></urls></record></Cite></EndNote>}. Chronic exposure to cannabinoids

is considered as a risk factor in the pathophysiology of various stress-related disorders such as depression, Posttraumatic stress disorder (PTSD) and other anxiety disorders { ADDIN EN.CITE

<EndNote><Cite><Author>Renard</Author><Year>2014</Year><RecNum>1550</RecNum><DisplayText>(6, 7)</DisplayText><record><rec-number>1550</rec-number><foreign-keys><key app="EN" db-id="50wxdpzd9vd5r7e9t5b595djrfpttrw9avp">1550</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Renard, Justine</author><author>Krebs, Marie-Odile</author><author>Le Pen, Gwenaëlle</author><author>Jay, Thérèse M</author></authors></contributors><titles><title>Long-term consequences of adolescent cannabinoid exposure in adult psychopathology</title><secondary-title>Frontiers in neuroscience</secondary-title></titles><periodical><full-title>Frontiers in Neuroscience</full-title><abbr-1>Front. Neurosci.</abbr-1><abbr-2>Front Neurosci</abbr-2></periodical><pages>361</pages><volume>8</volume><dates><year>2014</year></dates><isbn>1662-453X</isbn></urls></urls></record></Cite>

<Cite><Author>Panlilio</Author><Year>2018</Year><RecNum>1551</RecNum><record><rec-number>1551</rec-number><foreign-keys><key app="EN" db-id="50wxdpzd9vd5r7e9t5b595djrfpttrw9avp">1551</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Panlilio, Leigh V</author><author>Justinova, Zuzana</author></authors></contributors><titles><title>Preclinical Studies of Cannabinoid Reward, Treatments for Cannabis Use Disorder, and Addiction-Related Effects of Cannabinoid Exposure</title><secondary-title>Neuropsychopharmacology</secondary-title></titles><periodical><full-title>Neuropsychopharmacology</full-title><abbr-1>Neuropsychopharmacology</abbr-1><abbr-2>Neuropsychopharmacology</abbr-2></periodical><pages>116</pages><volume>43</volume><number>1</number><dates><year>2018</year></dates><isbn>1740-634X</isbn></urls></urls></record></Cite></EndNote>}. These psychotic issues are more common among cannabis users who began use at the age of adolescence (between 12 and 25 years old), an ontogenic stage during which the neuroendocrine system is still under development { ADDIN EN.CITE

<EndNote><Cite><Author>Romeo</Author><Year>2016</Year><RecNum>1552</RecNum>

m><DisplayText>(8, 9)</DisplayText><record><rec-number>1552</rec-number><foreign-keys><key app="EN" db-id="50wxdpzd9vd5r7e9t5b595djrfpttrw9avp">1552</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Romeo, Russell D</author><author>Patel, Ravenna</author><author>Pham, Laurie</author><author>So, Veronica M</author></authors></contributors><titles><title>Adolescence and the ontogeny of the hormonal stress response in male and female rats and mice</title><secondary-title>Neuroscience & Biobehavioral Reviews</secondary-title></titles><periodical><full-title>Neuroscience and Biobehavioral Reviews</full-title><abbr-1>Neurosci. Biobehav. Rev.</abbr-1><abbr-2>Neurosci Biobehav Rev</abbr-2><abbr-3>Neuroscience & Biobehavioral Reviews</abbr-3></periodical><pages>206-216</pages><volume>70</volume><dates><year>2016</year></dates><isbn>0149-7634</isbn><urls></urls></record></Cite><Cite><Author>Spear</Author><Year>2005</Year><RecNum>1553</RecNum><record><rec-number>1553</rec-number><foreign-keys><key app="EN" db-id="50wxdpzd9vd5r7e9t5b595djrfpttrw9avp">1553</key></foreign-keys><ref-type name="Book Section">5</ref-type><contributors><authors><author>Spear, Linda Patia</author><author>Varlinskaya, Elena I</author></authors></contributors><titles><title>Adolescence</title><secondary-title>Recent developments in alcoholism</secondary-title></titles><periodical><full-title>Recent Developments in Alcoholism</full-title><abbr-1>Recent Dev. Alcohol.</abbr-1><abbr-2>Recent Dev Alcohol</abbr-2></periodical><pages>143-159</pages><dates><year>2005</year></dates><publisher>Springer</publisher><urls></urls></record></Cite></EndNote>}. A recent study by Hill and Tasker reported a critical and complex role of the endocannabinoid system in glucocorticoid-mediated negative feedback, and regulation of the hypothalamic–pituitary–adrenal (HPA) axis activity. They revealed multiple sites of action by which a disruption in endocannabinoid signalling could result in an increase or decrease in HPA activity at multiple levels in distinct phases of stress response {

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<EndNote><Cite><Author>Bowers</Author><Year>2016</Year><RecNum>1523</RecNum><DisplayText>(10)</DisplayText><record><rec-number>1523</rec-number><foreign-keys><key app="EN" db-id="50wxdpzd9vd5r7e9t5b595djrfpttrw9avp">1523</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Bowers, Mallory

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Rachel</author></authors></contributors><titles><title>Intergenerational transmission of stress in humans</title><secondary-title>Neuropsychopharmacology</secondary-title></titles><periodical><full-title>Neuropsychopharmacology</full-title><abbr-1>Neuropsychopharmacology</abbr-1><abbr-2>Neuropsychopharmacology</abbr-2></periodical><pages>232</pages><volume>41</volume><number>1</number><dates><year>2016</year></dates><isbn>1740-

634X</isbn><urls></urls></record></Cite></EndNote>}. Also, it has been shown that chronic exposure to WIN during early adolescence in male rats cause long-lasting neurobiological changes that ultimately affect the emotional state of the adult brain { ADDIN EN.CITE { ADDIN EN.CITE.DATA }}. In humans, chronic cannabinoid exposure during adolescence has been associated with long-term structural and functional changes in the prefrontal cortex (PFC) and increased risk of developing psychotic disorders { ADDIN EN.CITE

<EndNote><Cite><Author>Renard</Author><Year>2014</Year><RecNum>6</RecNum><DisplayText>(6, 14)</DisplayText><record><rec-number>6</rec-number><foreign-keys><key app="EN" db-id="pxx2rpswxsaeteffz15a9vv9d29dxfava0f">6</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Renard, Justine</author><author>Krebs, Marie-Odile</author><author>Le Pen, Gwenaëlle</author><author>Jay, Thérèse M</author></authors></contributors><titles><title>Long-term consequences of adolescent cannabinoid exposure in adult psychopathology</title><secondary-title>Frontiers in neuroscience</secondary-title></titles><pages>361</pages><volume>8</volume><dates><year>2014</year></dates><isbn>1662-

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adolescence leads to long-term structural and functional changes in the prefrontal cortex

</title><secondary-title>European Neuropsychopharmacology</secondary-title></titles><periodical><full-title>European Neuropsychopharmacology</full-title><abbr-1>Eur. Neuropsychopharmacol.</abbr-1><abbr-2>Eur Neuropsychopharmacol</abbr-2></periodical><pages>55-64</pages><volume>26</volume><number>1</number><dates><year>2016</year></dates><isbn>0924-977X</isbn><urls></urls></record></Cite></EndNote>}.</p>
</div>
<div data-bbox="114 269 886 901" data-label="Text">
<p>On the other hand, the neurobiology of stress-related neuropsychiatric illness is complex, and the contributing problems are multifactorial { ADDIN EN.CITE</p>
<p><EndNote><Cite><Author>Lewis</Author><Year>2017</Year><RecNum>1497</RecNum><DisplayText>(15)</DisplayText><record><rec-number>1497</rec-number><foreign-keys><key app="EN" db-id="50wxdpzd9vd5r7e9t5b595djrfptrxw9avp">1497</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Lewis, Sian</author></authors></contributors><titles><title>Neuropsychiatric disorders: Stressful depression</title><secondary-title>Nature Reviews Neuroscience</secondary-title></titles><periodical><full-title>Nature Reviews Neuroscience</full-title></periodical><pages>3</pages><volume>19</volume><number>1</number><dates><year>2017</year></dates><isbn>1471-0048</isbn><urls></urls></record></Cite></EndNote>}. Paternal exposure to drugs of abuse might present one of the contributing factors to stress associated disorders { ADDIN EN.CITE</p>
<p><EndNote><Cite><Author>Porreca</Author><Year>2018</Year><RecNum>1498</RecNum><DisplayText>(16)</DisplayText><record><rec-number>1498</rec-number><foreign-keys><key app="EN" db-id="50wxdpzd9vd5r7e9t5b595djrfptrxw9avp">1498</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Porreca, Alessio</author><author>Biringen, Zeynep</author><author>Parolin, Micol</author><author>Saunders, Hannah</author><author>Ballarotto, Giulia</author><author>Simonelli, Alessandra</author></authors></contributors><titles><title>Emotional Availability, Neuropsychological Functioning, and Psychopathology: The Context of Parental Substance Use Disorder</title><secondary-title>BioMed research international</secondary-title></titles><periodical><full-title>BioMed research international</full-</p>
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title></periodical><volume>2018</volume><dates><year>2018</year></dates><isbn>2314-6133</isbn><urls></urls></record></Cite></EndNote>}. Indeed, recent works conducted on several animal and human generations has shown that the deleterious effects of drug abuse can pass down to subsequent generations through a transfer of epigenetic marks { ADDIN EN.CITE

<EndNote><Cite><Author>Ajonijebu</Author><Year>2017</Year><RecNum>1500</RecNum><DisplayText>(11)</DisplayText><record><rec-number>1500</rec-number><foreign-keys><key app="EN" db-id="50wxdpzd9vd5r7e9t5b595djrfpttrw9avp">1500</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Ajonijebu, Duyilemi C</author><author>Abboussi, Oualid</author><author>Russell, Vivienne A</author><author>Mabandla, Musa V</author><author>Daniels, William MU</author></authors></contributors><titles><title>Epigenetics: a link between addiction and social environment</title><secondary-title>Cellular and Molecular Life Sciences</secondary-title></titles><periodical><full-title>Cellular and Molecular Life Sciences</full-title><abbr-1>Cell. Mol. Life Sci.</abbr-1><abbr-2>Cell Mol Life Sci</abbr-2><abbr-3>Cellular & Molecular Life Sciences</abbr-3></periodical><pages>2735-2747</pages><volume>74</volume><number>15</number><dates><year>2017</year></dates><isbn>1420-682X</isbn><urls></urls></record></Cite></EndNote>}. The inherited physiological and behavioural traits observed in the offspring may differ and manifest at varying ages { ADDIN EN.CITE

<EndNote><Cite><Author>Patton</Author><Year>2018</Year><RecNum>1501</RecNum><DisplayText>(17)</DisplayText><record><rec-number>1501</rec-number><foreign-keys><key app="EN" db-id="50wxdpzd9vd5r7e9t5b595djrfpttrw9avp">1501</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Patton, George C</author><author>Olsson, Craig A</author><author>Skirbekk, Vegard</author><author>Saffery, Richard</author><author>Wlodek, Mary E</author><author>Azzopardi, Peter S</author><author>Stonawski, Marcin</author><author>Rasmussen, Bruce</author><author>Spry, Elizabeth</author><author>Francis, Kate</author></authors></contributors><titles><title>Adolescence and the next generation</title><secondary-title>Nature</secondary-title></titles><periodical><full-title>Nature</full-title><abbr-1>Nature</abbr-1><abbr-2>Nature</abbr-

2></periodical><pages>458</pages><volume>554</volume><number>7693</number><dates><year>2018</year></dates><isbn>1476-

4687</isbn><urls></urls></record></Cite></EndNote>}. These so-called transgenerational

epigenetic effects correspond to the mechanisms that control the expression of genes without

altering the DNA sequence { ADDIN EN.CITE

<EndNote><Cite><Author>Heard</Author><Year>2014</Year><RecNum>1503</RecNu

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2>Cell</abbr-2></periodical><pages>95-

109</pages><volume>157</volume><number>1</number><dates><year>2014</year></dat

es><isbn>0092-8674</isbn><urls></urls></record></Cite></EndNote>}. For instance, male

mice exposed to chronic intermittent ethanol prior to mating with ethanol naïve females

produce male offspring with reduced ethanol-drinking preference, increased ethanol

sensitivity, and increased brain-derived neurotrophic factor (BDNF) expression in the ventral

tegmental area (VTA) { ADDIN EN.CITE

<EndNote><Cite><Author>Rompala</Author><Year>2017</Year><RecNum>1504</RecN

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imparts intergenerational alcohol-related behaviors to male offspring on a pure C57BL/6J

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177</pages><volume>60</volume><dates><year>2017</year></dates><isbn>0741-

8329</isbn><urls></urls></record></Cite></EndNote>}. Also, it has been shown that paternal exposure to cocaine in rats result in epigenetic reprogramming of the germline that leads to profound effects on gene expression in the PFC and resistance to cocaine reinforcement in male offspring { ADDIN EN.CITE <EndNote><Cite><Author>Vassoler</Author><Year>2013</Year><RecNum>1505</RecNum><DisplayText>(20)</DisplayText><record><rec-number>1505</rec-number><foreign-keys><key app="EN" db-id="50wxdpzd9vd5r7e9t5b595djrfptrxw9avp">1505</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Vassoler, Fair M</author><author>White, Samantha L</author><author>Schmidt, Heath D</author><author>Sadri-Vakili, Ghazaleh</author><author>Pierce, R Christopher</author></authors></contributors><titles><title>Epigenetic inheritance of a cocaine-resistance phenotype</title><secondary-title>Nature neuroscience</secondary-title></titles><periodical><full-title>Nature Neuroscience</full-title><abbr-1>Nat. Neurosci.</abbr-1><abbr-2>Nat Neurosci</abbr-2></periodical><pages>42</pages><volume>16</volume><number>1</number><dates><year>2013</year></dates><isbn>1546-1726</isbn><urls></urls></record></Cite></EndNote>}. Moreover, Ali and al., have demonstrated that paternal stress exposure alters sperm microRNA content and dysregulates offspring HPA axis activity { ADDIN EN.CITE <EndNote><Cite><Author>Rodgers</Author><Year>2013</Year><RecNum>1507</RecNum><DisplayText>(21)</DisplayText><record><rec-number>1507</rec-number><foreign-keys><key app="EN" db-id="50wxdpzd9vd5r7e9t5b595djrfptrxw9avp">1507</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Rodgers, Ali B</author><author>Morgan, Christopher P</author><author>Bronson, Stefanie L</author><author>Revello, Sonia</author><author>Bale, Tracy L</author></authors></contributors><titles><title>Paternal stress exposure alters sperm microRNA content and reprograms offspring HPA stress axis regulation</title><secondary-title>Journal of Neuroscience</secondary-title></titles><periodical><full-title>Journal of Neuroscience</full-title><abbr-1>J. Neurosci.</abbr-1><abbr-2>J Neurosci</abbr-2></periodical><pages>9003-9012</pages><volume>33</volume><number>21</number><dates><year>2013</year></dates><isbn>0270-6474</isbn><urls></urls></record></Cite></EndNote>}. Unfortunately,

these animal observations concerning the inheritance of epigenetic marks doesn't translate into human studies due to the fact that male-line transgenerational studies are confounded by germlines susceptibility to social and environmental factors-induced epigenetic changes {
ADDIN EN.CITE

<EndNote><Cite><Author>Morgan</Author><Year>2008</Year><RecNum>1506</RecNum><DisplayText>(22, 23)</DisplayText><record><rec-number>1506</rec-number><foreign-keys><key app="EN" db-id="50wxdpzd9vd5r7e9t5b595djrfptrxw9avp">1506</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Morgan, Daniel K</author><author>Whitelaw, Emma</author></authors></contributors><titles><title>The case for transgenerational epigenetic inheritance in humans</title><secondary-title>Mammalian Genome</secondary-title></titles><periodical><full-title>Mammalian Genome</full-title><abbr-1>Mamm. Genome</abbr-1><abbr-2>Mamm Genome</abbr-2></periodical><pages>394-397</pages><volume>19</volume><number>6</number><dates><year>2008</year></date><isbn>0938-8990</isbn></urls></record></Cite><Cite><Author>Walker</Author><Year>2018</Year><RecNum>1508</RecNum><record><rec-number>1508</rec-number><foreign-

keys><key app="EN" db-id="50wxdpzd9vd5r7e9t5b595djrfptrxw9avp">1508</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Walker, Vickie R</author><author>Boyles, Abee L</author><author>Pelch, Katherine E</author><author>Holmgren, Stephanie D</author><author>Shapiro, Andrew J</author><author>Blystone, Chad R</author><author>Devito, Michael J</author><author>Newbold, Retha R</author><author>Blain, Robyn</author><author>Hartman, Pamela</author></authors></contributors><titles><title>Human and animal evidence of potential transgenerational inheritance of health effects: An evidence map and state-of-the-science evaluation</title><secondary-title>Environment international</secondary-title></titles><periodical><full-title>Environment international</full-title></periodical><pages>48-69</pages><volume>115</volume><dates><year>2018</year></dates><isbn>0160-4120</isbn></urls></record></Cite></EndNote>}.To date, the precise mechanisms explaining the inheritance of behavioural and physiological traits are still ambiguous in

particular in the context of paternal cannabinoids abuse { ADDIN EN.CITE <EndNote><Cite><Author>ZHANG</Author><Year>2017</Year><RecNum>1509</RecNum><DisplayText>(24)</DisplayText><record><rec-number>1509</rec-number><foreign-keys><key app="EN" db-id="50wxdpzd9vd5r7e9t5b595djrfpttrw9avp">1509</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>ZHANG, Ke</author><author>DUAN, Ying</author></authors></contributors><titles><title>The impact of the parental preconception exposure to addictive drugs on the offsprings' behavior and the epigenetic mechanism</title><secondary-title>Advances in Psychological Science</secondary-title></titles><periodical><full-title>Advances in Psychological Science</full-title></periodical><pages>1791-1798</pages><volume>25</volume><number>10</number><dates><year>2017</year></dates><isbn>1671-3710</isbn><urls></urls></record></Cite></EndNote>}. Nevertheless, several epigenetic changes have been correlated to dysregulated gene expression pattern in animal models of chronic exposure to cannabinoids during adolescence, such as DNA methylation, histone modifications and RNA interference { ADDIN EN.CITE { ADDIN EN.CITE.DATA }}. Also, it has been shown that some epigenetic changes can escape the epigenomic reprogramming that occurs during gametogenesis and fertilization and affects behaviour and vulnerability to stress in subsequent generations { ADDIN EN.CITE { ADDIN EN.CITE.DATA }}. Moreover, unlike RNA interference and histone modifications, DNA methylation is presumably a more static epigenetic mark and possibly transgenerationally transferable { ADDIN EN.CITE <EndNote><Cite><Author>Williams</Author><Year>2017</Year><RecNum>1515</RecNum><DisplayText>(30)</DisplayText><record><rec-number>1515</rec-number><foreign-keys><key app="EN" db-id="50wxdpzd9vd5r7e9t5b595djrfpttrw9avp">1515</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Williams, Ben P</author><author>Gehring, Mary</author></authors></contributors><titles><title>Stable transgenerational epigenetic inheritance requires a DNA methylation-sensing circuit</title><secondary-title>Nature communications</secondary-title></titles><periodical><full-title>Nature communications</full-title></periodical><pages>2124</pages><volume>8</volume><number>1</number><dates><year>2017</year></dates><isbn>2041-1723</isbn><urls></urls></record></Cite></EndNote>}. DNA methylation is mediated by

DNA methyltransferase enzymes DNMT1, DNMT3a and DNMT3b { ADDIN EN.CITE <EndNote><Cite><Author>Du</Author><Year>2015</Year><RecNum>1517</RecNum><DisplayText>(31)</DisplayText><record><rec-number>1517</rec-number><foreign-keys><key app="EN" db-id="50wxdpzd9vd5r7e9t5b595djrfptrxw9avp">1517</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Du, Jiamu</author><author>Johnson, Lianna M</author><author>Jacobsen, Steven E</author><author>Patel, Dinshaw J</author></authors></contributors><titles><title>DNA methylation pathways and their crosstalk with histone methylation</title><secondary-title>Nature Reviews Molecular Cell Biology</secondary-title></titles><periodical><full-title>Nature Reviews Molecular Cell Biology</full-title></periodical><pages>519</pages><volume>16</volume><number>9</number><dates><year>2015</year></dates><isbn>1471-0080</isbn><urls></urls></record></Cite></EndNote>}. DNMT1 and DNMT3a have been proved substantial for maintenance and de novo methylation of DNA, respectively. Both are expressed in postmitotic neurons and contribute to adaptive neuronal gene expression in response to environmental stimuli including emotional regulation { ADDIN EN.CITE <EndNote><Cite><Author>Du</Author><Year>2015</Year><RecNum>1517</RecNum><DisplayText>(31)</DisplayText><record><rec-number>1517</rec-number><foreign-keys><key app="EN" db-id="50wxdpzd9vd5r7e9t5b595djrfptrxw9avp">1517</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Du, Jiamu</author><author>Johnson, Lianna M</author><author>Jacobsen, Steven E</author><author>Patel, Dinshaw J</author></authors></contributors><titles><title>DNA methylation pathways and their crosstalk with histone methylation</title><secondary-title>Nature Reviews Molecular Cell Biology</secondary-title></titles><periodical><full-title>Nature Reviews Molecular Cell Biology</full-title></periodical><pages>519</pages><volume>16</volume><number>9</number><dates><year>2015</year></dates><isbn>1471-0080</isbn><urls></urls></record></Cite></EndNote>}. Recent evidence has suggested that abnormal patterns of DNMTs expression could be involved in the aberrant gene expression observed in stressed animals as well as in patients with mood disorders { ADDIN EN.CITE <EndNote><Cite><Author>Blaze</Author><Year>2015</Year><RecNum>1518</RecNum><DisplayText>(32)</DisplayText><record><rec-number>1518</rec-number><foreign-

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 Jennifer</author><author>Roth, Tania
 L</author></authors></contributors><titles><title>Evidence from clinical and animal model
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 Cell Dev Biol</abbr-2><abbr-3>Seminars in Cell & Developmental Biology</abbr-
 3></periodical><pages>76-
 84</pages><volume>43</volume><dates><year>2015</year></dates><publisher>Elsevier<
 /publisher><isbn>1084-9521</isbn><urls></urls></record></Cite></EndNote>}. It has been
 shown that inhibition of DNA methylation in the hippocampus as well as in the nucleus
 accumbens induces antidepressant-like effects, decreases DNA methylation and increases
 brain-derived neurotrophic factor (BDNF) levels in the hippocampus { ADDIN EN.CITE
 <EndNote><Cite><Author>Sales</Author><Year>2011</Year><RecNum>1519</RecNum
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 J</author><author>Biojone, Caroline</author><author>Terceti, Mateus
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 RL</author></authors></contributors><titles><title>Antidepressant-like effect induced by
 systemic and intra-hippocampal administration of DNA methylation
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 J. Pharmacol.</abbr-1><abbr-2>Br J Pharmacol</abbr-2></periodical><pages>1711-
 1721</pages><volume>164</volume><number>6</number><dates><year>2011</year></d
 ates><isbn>0007-1188</isbn><urls></urls></record></Cite></EndNote>}. Also, recent
 evidence has suggested that exposure to social and environmental stress increases the
 expression of DNMTs in different brain regions including the PFC, thus increasing DNA

methylation and decreasing the expression of genes implicated in synaptic plasticity and neurotransmission { ADDIN EN.CITE <EndNote><Cite><Author>Sales</Author><Year>2018</Year><RecNum>1520</RecNum><DisplayText>(34)</DisplayText><record><rec-number>1520</rec-number><foreign-keys><key app="EN" db-id="50wxdpzd9vd5r7e9t5b595djrfpttrxw9avp">1520</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Sales, Amanda J</author><author>Joca, Sâmia RL</author></authors></contributors><titles><title>Antidepressant administration modulates stress-induced DNA methylation and DNA methyltransferase expression in rat prefrontal cortex and hippocampus</title><secondary-title>Behavioural brain research</secondary-title></titles><periodical><full-title>Behavioural Brain Research</full-title><abbr-1>Behav. Brain Res.</abbr-1><abbr-2>Behav Brain Res</abbr-2></periodical><pages>8-15</pages><volume>343</volume><dates><year>2018</year></dates><isbn>0166-4328</isbn><urls></urls></record></Cite></EndNote>}. Similarly, a number of studies in humans on childhood trauma and post-traumatic stress disorder demonstrated long-term changes in global and regional DNA methylation profiles { ADDIN EN.CITE { ADDIN EN.CITE.DATA }}.

Taken together, these studies suggest that chronic cannabinoid exposure is likely to induce transgenerational effects that can influence stress responses in the offspring. Thus in the present work, we aimed to determine if chronic exposure to WIN during adolescence in male rats would induce vulnerability to stress-induced emotional and cognitive alterations in the offspring, and whether it may alter global DNA methylation and its related enzymes DNMT1 and DNMT3a transcription.

2. Material and methods

2.1. Animals

Male Wistar rats (21 days old) were obtained from the animal facility of the University Mohammed V (Morocco). They were housed in groups of four per cage (30.80 x 30.80 x 18.72 (cm.)), kept at a constant temperature (22 ± 2 °C) under a 12/12 h light-dark cycle (light beginning at 7:00 a.m.) and were provided with food and water *ad libitum*.

All animals were maintained in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). All procedures were approved by the Animal Ethics Committee of the University Mohammed V.

At 30 days of age, animals received, during 20 days, daily intraperitoneal injections of vehicle or WIN55,212-2 (1.2 mg/kg) (Tocris Bioscience, France) dissolved in 0.1 % Tween 80 and diluted in saline 0.9 %. They were injected 10 times one injection, five times two injections and 10 times no injection, alternately, to model human use patterns { ADDIN EN.CITE

<EndNote><Cite><Author>Abboussi</Author><Year>2016</Year><RecNum>1524</RecNum><DisplayText>(12, 37)</DisplayText><record><rec-number>1524</rec-number><foreign-keys><key app="EN" db-id="50wxdpzd9vd5r7e9t5b595djrfpttrw9avp">1524</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Abboussi, Oualid</author><author>Said, Nadia</author><author>Fifel, Karim</author><author>Lakehayli, Sara</author><author>Tazi, Abdelouahhab</author><author>El Ganouni, Soumaya</author></authors></contributors><titles><title>Behavioral effects of D3 receptor inhibition and 5-HT4 receptor activation on animals undergoing chronic cannabinoid exposure during adolescence</title><secondary-title>Metabolic brain disease</secondary-title></titles><periodical><full-title>Metabolic Brain Disease</full-title><abbr-1>Metab. Brain Dis.</abbr-1><abbr-2>Metab Brain Dis</abbr-2></periodical><pages>321-327</pages><volume>31</volume><number>2</number><dates><year>2016</year></date s><isbn>0885-

7490</isbn><urls></urls></record></Cite><Cite><Author>Schneider</Author><Year>2007</Year><RecNum>1525</RecNum><record><rec-number>1525</rec-number><foreign-keys><key app="EN" db-id="50wxdpzd9vd5r7e9t5b595djrfpttrw9avp">1525</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Schneider, Miriam</author><author>Koch,

Michael</author></authors></contributors><titles><title>The effect of chronic peripubertal cannabinoid treatment on deficient object recognition memory in rats after neonatal mPFC lesion</title><secondary-title>European Neuropsychopharmacology</secondary-title></titles><periodical><full-title>European Neuropsychopharmacology</full-title><abbr-1>Eur. Neuropsychopharmacol.</abbr-1><abbr-2>Eur Neuropsychopharmacol</abbr-

2></periodical><pages>180-186</pages><volume>17</volume><number>3</number><dates><year>2007</year></date s><isbn>0924-977X</isbn><urls></urls></record></Cite></EndNote>} (Figure 1).

20 day following the last WIN injection, each male rat was placed in a breeding cage with 2 untreated females (75 days old). When the females were visibly pregnant they were singly housed. Their litters were culled to 7 pups on postnatal day 1 and weaned at 21 days of age. The total male progeny generated was 32 rats. They were group housed in standard conditions with same-sex until adulthood (60 days old).

2.2. Unpredictable stress regimen

Adult offspring of non-exposed fathers (NF) (n=8) and offspring of WIN exposed fathers (WF) (60 days old) (n=8) were subjected to unpredictable variable stress for one week (Figure 1). Stress regimen consisted of exposure, once daily, to one of the following stressors: restraint stress for 6 h; warm swim (22 °C) in a circular basin (120 cm in diameter and 40 cm in deep) for 15 min; shaking (groups of four rats were placed in a plastic box container and placed in an orbital shaker for 1 h at 150 rpm); food and water deprivation for 24 h; constant light for 24 h; tilted cage (home cages were tilted in a 45° angle three times 30 min at 1 h intervals); crowding (8 males in a cage 55x30x20 cm high) with constant light for 24 h { ADDIN EN.CITE <EndNote><Cite><Author>Jesse</Author><Year>2015</Year><RecNum>1569</RecNum>><DisplayText>(38)</DisplayText><record><rec-number>1569</rec-number><foreign-keys><key app="EN" db-id="50wxdpzd9vd5r7e9t5b595djrfpttrxw9avp">1569</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Jesse, CR</author><author>Donato, F</author><author>Giacomeli, R</author><author>Del Fabbro, L</author><author>da Silva Antunes, M</author><author>de Gomes, MG</author><author>Goes, ATR</author><author>Boeira, SP</author><author>Prigol, M</author><author>Souza, LC</author></authors></contributors><titles><title>Chronic unpredictable mild stress decreases BDNF and NGF levels and Na⁺, K⁺-ATPase activity in the hippocampus and prefrontal cortex of mice: Antidepressant effect of chrysin</title><secondary-title>Neuroscience</secondary-title></titles><periodical><full-title>Neuroscience</full-title><abbr-1>Neuroscience</abbr-1><abbr-2>Neuroscience</abbr-2></periodical><pages>367-380</pages><volume>289</volume><dates><year>2015</year></dates><isbn>0306-

4522</isbn><urls></urls></record></Cite></EndNote>}. During this period animals of the control groups, NF (n=8) and WF (n=8) were left undisturbed, except for cleaning the cages.

2.3. Behavioural tests

All behavioural tests were conducted 24 hours after the last stress session, video-recorded, tracked, and analysed later. The experimenter was unaware of the treatment allocation from the start of the experiment until after the results have been analysed to guard against the experimenter bias.

2.3.1. The Open field test

We tested all animals (n=32) at the age of 68 postnatal days in an open field, using a grey Plexiglas square box (1 m x 1 m x 0.5 m). Its floor was divided into 25 equal squares of 20 x 20 cm each, 16 peripherals and 9 centrals, and was brightly illuminated (70 lux). The animals were tested and video recorded for 5 min. The test was started by placing the animal in the open field facing the corner. Two parameters were analysed, the time spent by the animal in the central squares as a measure of anxiety-like behaviour and the total number of squares crossed as a measure of locomotor activity { ADDIN EN.CITE <EndNote><Cite><Author>Prut</Author><Year>2003</Year><RecNum>1570</RecNum><DisplayText>(39)</DisplayText><record><rec-number>1570</rec-number><foreign-keys><key app="EN" db-id="50wxdpzd9vd5r7e9t5b595djrfpttrxw9avp">1570</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Prut, Laetitia</author><author>Belzung, Catherine</author></authors></contributors><titles><title>The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review</title><secondary-title>European journal of pharmacology</secondary-title></titles><periodical><full-title>European Journal of Pharmacology</full-title><abbr-1>Eur. J. Pharmacol.</abbr-1><abbr-2>Eur J Pharmacol</abbr-2></periodical><pages>3-33</pages><volume>463</volume><number>1-3</number><dates><year>2003</year></dates><isbn>0014-2999</isbn><urls></urls></record></Cite></EndNote>}.</p>
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2.3.2. The Novel object recognition test

The object recognition task was conducted to evaluate the ability of the animals to discriminate between novel and familiar objects. We used the same apparatus as in the open field test. The light was adjusted to 15 lux to avoid any stress from bright light. The test procedure consisted of three sessions: familiarization, acquisition, and retention. During the first day, rats were familiarized to the apparatus for 5 min. During the second day (acquisition session), two objects (A1 and A2) were placed 0.5 m from the sidewall in diagonal corners opposite each other. The rat was placed in the corner of the apparatus facing the objects and allowed to explore the objects freely until 20 seconds of exploration for each object is reached or when a 10 min period is over (the maximum session time) than the rat was removed from the apparatus and returned to its home cage. After 24 h inter-trial interval, the rat was returned to the NORT apparatus (retention session) that contained the familiar object used during the acquisition session and a new object and allowed 5 min of exploration. Objects of various shapes ranging in size from about $10 \times 9 \times 8$ cm to $12 \times 10 \times 13$ cm were used. All objects and the apparatus were cleaned using 70 % ethanol to eliminate olfactory stimuli. Objects were randomized between each rat and each group tested. Duration of approaching (< 1cm distance), touching or sniffing the objects were scored. The discrimination index (DI) was calculated (time spent exploring the novel object - time spent exploring the familiar object)/total exploration time { ADDIN EN.CITE

<EndNote><Cite><Author>Bevins</Author><Year>2006</Year><RecNum>1526</RecNum><DisplayText>(40)</DisplayText><record><rec-number>1526</rec-number><foreign-keys><key app="EN" db-id="50wxdpzd9vd5r7e9t5b595djrfpttrxw9avp">1526</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Bevins, Rick A</author><author>Besheer, Joyce</author></authors></contributors><titles><title>Object recognition in rats and mice: a one-trial non-matching-to-sample learning task to study'recognition memory'</title><secondary-title>Nature protocols</secondary-title></titles><periodical><full-title>Nature Protocols</full-title><abbr-1>Nat. Protoc.</abbr-1><abbr-2>Nat Protoc</abbr-2></periodical><pages>1306</pages><volume>1</volume><number>3</number><dates><year>2006</year></dates><isbn>1750-2799</isbn><urls></urls></record></Cite></EndNote>}.</p></div>

2.4. Plasma and tissue Collection

24 h after the last behavioural test, animals were decapitated and trunk blood was collected into EDTA tubes and centrifuged at 3000 x g for 10 min to separate the plasma. The plasma and the prefrontal cortex dissected from the animal brains were snap frozen in liquid nitrogen and stored in a bio-freezer at -80°C for biochemical analysis.

2.5. Determination of plasma corticosterone levels

The corticosterone levels in the plasma were measured using a commercially available enzyme immunoassay kit (corticosterone ELISA, IBL-International, UK) according to the manufacturer's instructions { ADDIN EN.CITE <EndNote><Cite><Author>Murakami</Author><Year>2005</Year><RecNum>1571</RecNum><DisplayText>(41)</DisplayText><record><rec-number>1571</rec-number><foreign-keys><key app="EN" db-id="50wxdpzd9vd5r7e9t5b595djrfptrxw9avp">1571</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Murakami, Shuji</author><author>Imbe, Hiroki</author><author>Morikawa, Yoshihiro</author><author>Kubo, Chiharu</author><author>Senba, Emiko</author></authors></contributors><titles><title>Chronic stress, as well as acute stress, reduces BDNF mRNA expression in the rat hippocampus but less robustly</title><secondary-title>Neuroscience Research</secondary-title></titles><periodical><full-title>Neuroscience Research</full-title><abbr-1>Neurosci. Res.</abbr-1><abbr-2>Neurosci Res</abbr-2></periodical><pages>129-139</pages><volume>53</volume><number>2</number><keywords><keyword>Restraint</keyword><keyword>HPA axis</keyword><keyword>Glucocorticoid receptor</keyword><keyword>5-HT</keyword><keyword>Tryptophan hydroxylase</keyword><keyword>Median raphe nucleus</keyword></keywords><dates><year>2005</year><pub-dates><date>2005/10/01</date></pub-dates></dates><isbn>0168-0102</isbn><urls><related-urls><url><http://www.sciencedirect.com/science/article/pii/S0168010205001641></url></related-urls></urls><electronic-resource-num><https://doi.org/10.1016/j.neures.2005.06.008></electronic-resource-num></record></Cite></EndNote>}.</p></div>

2.6. Determination of global DNA methylation

For global DNA methylation, the genomic DNA was first purified from the PFC tissue using a QIAamp DNA kit (Qiagen, UK), then 100 ng of DNA from each sample was examined by a 5-mC DNA Methylation ELISA kit (ZYMO Research, UK) in triplicates following the manufacturer's instructions { ADDIN EN.CITE <EndNote><Cite><Author>Makhathini</Author><Year>2017</Year><RecNum>1572</RecNum><DisplayText>(42)</DisplayText><record><rec-number>1572</rec-number><foreign-keys><key app="EN" db-id="50wxdpzd9vd5r7e9t5b595djrfpttrw9avp">1572</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Makhathini, Khayelihle B.</author><author>Abboussi, Oualid</author><author>Stein, Dan J.</author><author>Mabandla, Musa V.</author><author>Daniels, William M. U.</author></authors></contributors><titles><title>Repetitive stress leads to impaired cognitive function that is associated with DNA hypomethylation, reduced BDNF and a dysregulated HPA axis</title><secondary-title>International Journal of Developmental Neuroscience</secondary-title></titles><periodical><full-title>International Journal of Developmental Neuroscience</full-title><abbr-1>Int. J. Dev. Neurosci.</abbr-1><abbr-2>Int J Dev Neurosci</abbr-2></periodical><pages>63-69</pages><volume>60</volume><keywords><keyword>Repetitive stress</keyword><keyword>HPA axis</keyword><keyword>DNA methylation</keyword><keyword>Hippocampus</keyword><keyword>Cognitive behavior</keyword><keyword>Anxiety</keyword></keywords><dates><year>2017</year><pub-dates><date>2017/08/01</date></pub-dates></dates><isbn>0736-5748</isbn><urls><related-urls><url><http://www.sciencedirect.com/science/article/pii/S0736574816302842></url></related-urls></urls><electronic-resource-num><https://doi.org/10.1016/j.ijdevneu.2017.04.004></electronic-resource-num></record></Cite></EndNote>}.

2.7. Determination of DNMT1 and DNMT3a expression

Real-time polymerase chain reaction (RT-PCR) was used to measure gene expression in PFC tissue. Total RNA was first extracted using RNeasy Mini Kit (Qiagen, UK). 2 µg RNA from each sample was treated with Dnase I (Invitrogen) and reverse transcription was performed

using iScript™ cDNA Synthesis Kit (Bio-RAD, UK) as described by the manufacturers. In a final volume of 20 µl, 5 µl per well of cDNA was used along with 3 µl of deionised water, 10 µl of SYBR Green and 2 µl of each of the following primers (Forward: 5'-TTGTGGTGAGCCAGGTAGAAAGT-3', Reverse: 3'- AAGAAGATGGGCGTCTCATCA-5' for DNMT1; Forward: 5'- AAACGGAAGCGGGATGAGT-3', Reverse: 3'-TACTGCAATCACCTTGGCTTTC-5' for DNMT3a and Forward: 5'-ACCCTAAGGCCAACCTGAAA-3' Reverse: 3'-TCATTGCCGATAGTGATGACCTGAC-5' for β-actin serving as a reference gene). The PCR was performed using Roche Light Cycler® 480 real-time PCR system (Roche Diagnostics, USA): 35 cycles of DNA denaturation (5 min at 95 °C), primer hybridization (30 s at 95 °C), elongation (30 s at 72 °C) and a final elongation step (7 min at 72 °C) { ADDIN EN.CITE <EndNote><Cite><Author>Feng</Author><Year>2010</Year><RecNum>1574</RecNum><DisplayText>(43)</DisplayText><record><rec-number>1574</rec-number><foreign-keys><key app="EN" db-id="50wxdpzd9vd5r7e9t5b595djrfpttrxw9avp">1574</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Feng, Jian</author><author>Zhou, Yu</author><author>Campbell, Susan L</author><author>Le, Thuc</author><author>Li, En</author><author>Sweatt, J David</author><author>Silva, Alcino J</author><author>Fan, Guoping</author></authors></contributors><titles><title>Dnmt1 and Dnmt3a maintain DNA methylation and regulate synaptic function in adult forebrain neurons</title><secondary-title>Nature neuroscience</secondary-title></titles><periodical><full-title>Nature Neuroscience</full-title><abbr-1>Nat. Neurosci.</abbr-1><abbr-2>Nat Neurosci</abbr-2></periodical><pages>423</pages><volume>13</volume><number>4</number><dates><year>2010</year></dates><isbn>1546-1726</isbn></urls></record></Cite></EndNote>}. Samples were analysed in triplicates to make sure products from all PCR runs were cross-comparable and a melting curve analysis was done to detect the presence of non-specific products. To determine fold changes, we used the $\Delta\Delta CT$ method { ADDIN EN.CITE <EndNote><Cite><Author>Livak</Author><Year>2001</Year><RecNum>1573</RecNum><DisplayText>(44)</DisplayText><record><rec-number>1573</rec-number><foreign-keys><key app="EN" db-id="50wxdpzd9vd5r7e9t5b595djrfpttrxw9avp">1573</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Livak, Kenneth

J</author><author>Schmittgen, Thomas
D</author></authors></contributors><titles><title>Analysis of relative gene expression data
using real-time quantitative PCR and the 2- $\Delta\Delta$ CT method</title><secondary-
title>methods</secondary-title></titles><periodical><full-title>Methods</full-title><abbr-
1>Methods</abbr-1><abbr-2>Methods</abbr-2></periodical><pages>402-
408</pages><volume>25</volume><number>4</number><dates><year>2001</year></date
s><isbn>1046-2023</isbn><urls></urls></record></Cite></EndNote>}.

2.8. Statistical analyses

All results are reported as the mean \pm SEM (standard error of mean). Data were analysed using GraphPad Prism (version 5, San Diego, California, USA). All results were tested for normality using Shapiro-Wilk test followed by a two-way ANOVA with paternal exposure to WIN and stress as factors. Significant main effects were followed by Bonferroni *post-hoc* test. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Open field test

The offspring of WF and NF were subjected to the open field test to assess anxiety-like behaviour. Two parameters were measured in this test, the number of line crossings and the time spent in the centre of the open field. Two-way ANOVA analysis indicated no significant effect of paternal exposure to WIN ($F(1,28)=0.289$; $p=0.595$) or stress ($F(1,28)=3.599$; $p=0.0682$) on locomotor activity as expressed by the number of line crossings (Figure 2). However, when analysing anxiety-like behaviour as expressed by the time spent by offspring in the centre of the open field, two-ANOVA analysis revealed a main effect of paternal exposure to WIN ($F(1,28)=11.81$; $p=0.002$) and stress ($F(1,28)=19.78$; $p=0.0001$) with a significant interaction between these two factors ($F(1,28)=4.946$; $p=0.0344$). Further analysis with Bonferroni *post-hoc* test showed a significant decrease in the time spent in the centre of the open field arena in the offspring of WF in comparison to the offspring of NF when subjected to unpredictable stress ($p<0.001$) (Figure 3).

3.2. Novel object recognition test

Episodic-like memory in the offspring was evaluated using the novel object recognition test. Two-way ANOVA analysis revealed no effect of paternal exposure to WIN ($F(1,28)=1.766$; $p=0.194$) or stress ($F(1,28)=2.621$; $p=0.117$) on objects discrimination in the offspring (Figure 4).

3.3. Plasma corticosterone level

Two-way ANOVA analysis revealed an increased plasma { [HYPERLINK "https://www.sciencedirect.com/topics/neuroscience/corticosterone"](https://www.sciencedirect.com/topics/neuroscience/corticosterone) \o "Learn more about Corticosterone" } level in the offspring of WF and NF when subjected to stress ($F(1,28)=30.28$; $p<0.001$) and no effect of paternal exposure to WIN ($F(1,28)=2.083$; $p=0.16$) (Figure 5).

3.4. PFC global DNA methylation

Statistical analysis of global DNA methylation in the PFC using 5-mC DNA ELISA demonstrated a main effect of stress ($F(1,28)=19.90$, $p<0.001$) and a significant interaction between stress and paternal exposure to WIN ($F(1,28)=7.896$, $p=0.0089$). *Post-hoc* analysis demonstrated a significant increase in global methylation as expressed by increased 5-mc percentage in the offspring of WF in comparison to the offspring of NF when exposed to unpredicted stress $p<0.05$ (Figure 6).

3.5. DNMT1 and DNMT3a mRNA expression in the PFC

To investigate the mechanism by which unpredictable stress may increase DNA methylation in the offspring of WF, we examined the expression of DNMT1 and DNMT3a mRNA in the PFC. The obtained results showed a significant effect of paternal exposure to WIN ($F(1,28)=8.217$, $p=0.0078$) and stress ($F(1,28)=5.347$, $p=0.0283$) on DNMT1 mRNA expression in the PFC of the offspring with no significant interaction ($F(1,28)=2.243$, $p=0.145$). Bonferroni *post-hoc* comparisons showed an increased DNMT1 mRNA level in the offspring of WF in comparison to NF ($p<0.01$) in non-stressed animals, and increased DNMT1 mRNA level in, both, the offspring of WF and NF when subjected to unpredicted stress when compared to non-stressed offspring of NF ($p<0.05$) (Figure 7A). However, Two-way ANOVA analysis showed a significant effect of paternal exposure to WIN on DNMT3a mRNA expression ($F(1,28)=4.973$, $p=0.034$) and no effect of stress exposure ($F(1,28)=0.515$, $p=0.478$). *Post-hoc* comparisons showed an increased DNMT3a mRNA expression in the PFC of the offspring of WF when compared to the offspring of NF ($p<0.05$) (Figure 7B).

4. Discussion

While it is well-known that cannabinoids abuse during adolescence can confer long-lasting neurobiological and behavioural alterations in adulthood. Investigators have only recently begun to assess underlying epigenetic modifications and its inheritance to subsequent generations. We have previously shown that chronic exposure to WIN during adolescence induces anxiety and memory deficits and decreases neuronal plasticity in rats { ADDIN EN.CITE

<EndNote><Cite><Author>Abboussi</Author><Year>2016</Year><RecNum>1524</RecNum><DisplayText>(12, 13)</DisplayText><record><rec-number>1524</rec-number><foreign-keys><key app="EN" db-id="50wxdpzd9vd5r7e9t5b595djrfpttrw9avp">1524</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Abboussi, Oualid</author><author>Said, Nadia</author><author>Fifel, Karim</author><author>Lakehayli, Sara</author><author>Tazi, Abdelouahhab</author><author>El Ganouni, Soumaya</author></authors></contributors><titles><title>Behavioral effects of D3 receptor inhibition and 5-HT4 receptor activation on animals undergoing chronic cannabinoid exposure during adolescence</title><secondary-title>Metabolic brain disease</secondary-title></titles><periodical><full-title>Metabolic Brain Disease</full-title><abbr-1>Metab. Brain Dis.</abbr-1><abbr-2>Metab Brain Dis</abbr-2></periodical><pages>321-327</pages><volume>31</volume><number>2</number><dates><year>2016</year></date s><isbn>0885-

7490</isbn><urls></urls></record></Cite><Cite><Author>Abboussi</Author><Year>2014</Year><RecNum>1527</RecNum><record><rec-number>1527</rec-number><foreign-keys><key app="EN" db-id="50wxdpzd9vd5r7e9t5b595djrfpttrw9avp">1527</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Abboussi, Oualid</author><author>Tazi, Abdelouahhab</author><author>Paizanis, Eleni</author><author>El Ganouni, Soumaya</author></authors></contributors><titles><title>Chronic exposure to WIN55, 212-2 affects more potently spatial learning and memory in adolescents than in adult rats via a negative action on dorsal hippocampal neurogenesis</title><secondary-title>Pharmacology Biochemistry and Behavior</secondary-title></titles><periodical><full-title>Pharmacology

Biochemistry and Behavior</full-title></periodical><pages>95-102</pages><volume>120</volume><dates><year>2014</year></dates><isbn>0091-3057</isbn><urls></urls></record></Cite></EndNote>}. Also, it has been shown that chronic cannabinoid exposure in adolescent female rats increases anxiety and drug seeking behaviour in the offspring { ADDIN EN.CITE <EndNote><Cite><Author>Byrnes</Author><Year>2012</Year><RecNum>1528</RecNum><DisplayText>(45)</DisplayText><record><rec-number>1528</rec-number><foreign-keys><key app="EN" db-id="50wxdpzd9vd5r7e9t5b595djrfpttrw9avp">1528</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Byrnes, John J</author><author>Johnson, Nicole L</author><author>Schenk, Marian E</author><author>Byrnes, Elizabeth M</author></authors></contributors><titles><title>Cannabinoid exposure in adolescent female rats induces transgenerational effects on morphine conditioned place preference in male offspring</title><secondary-title>Journal of psychopharmacology</secondary-title></titles><periodical><full-title>Journal of psychopharmacology</full-title></periodical><pages>1348-1354</pages><volume>26</volume><number>10</number><dates><year>2012</year></dates><isbn>0269-8811</isbn><urls></urls></record></Cite></EndNote>}. However, to date, no study has examined the effects of paternal exposure to cannabinoids on offspring behaviour, in particular, resilience to stress. Thus, in the present study we examined the behavioural and epigenetic effects of paternal exposure to cannabinoids during adolescence on male offspring.

Using a rat model of unpredicted stress, we demonstrate for the first time that chronic exposure to cannabinoids during adolescence prone male offspring to stress-induced anxiety-like behaviour, increases global PFC DNA methylation through upregulation of DNA methyltransferase enzymes DNMT1 and DNMT3a and had no significant effect on cognitive functions.

Chronic unpredictable stress can have deleterious consequences on behaviour as well as neuroendocrine and autonomic functions. It has repeatedly shown that exposure to unpredictable stress for 14 consecutive days increases DNA methylation, decreases the expression of genes involved in neural plasticity and induces cognitive deficits and anxiety-

like behaviour in rats { ADDIN EN.CITE { ADDIN EN.CITE.DATA }}. It has also been demonstrated that susceptibility and resilience to stress might vary according to stress amplitude, sex, genetic background and/or early life experience { ADDIN EN.CITE

<EndNote><Cite><Author>Pereira</Author><Year>2017</Year><RecNum>1533</RecNum><DisplayText>(50)</DisplayText><record><rec-number>1533</rec-number><foreign-keys><key app="EN" db-id="50wxdpzd9vd5r7e9t5b595djrfptrxw9avp">1533</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Pereira, Vitor H</author><author>Campos, Isabel</author><author>Sousa, Nuno</author></authors></contributors><titles><title>The role of autonomic nervous system in susceptibility and resilience to stress</title><secondary-title>Current Opinion in Behavioral Sciences</secondary-title></titles><periodical><full-title>Current Opinion in Behavioral Sciences</full-title></periodical><pages>102-107</pages><volume>14</volume><dates><year>2017</year></dates><isbn>2352-1546</isbn><urls></urls></record></Cite></EndNote>}. In the present study, using 7 consecutive days of unpredictable stress regimen, we found that male offspring of WF display increased anxiety in comparison to the offspring of NF. Interestingly, although the offspring of WF and NF exhibited two distinct behavioural phenotypes (susceptible and resilient to stress) both demonstrated a significant increase in plasmatic corticosterone levels in response to stress which may reflect a disturbance in neuroendocrine signalling in the offspring of WF in particular expression and/or function of corticosterone receptors (e.g mineralocorticoid and glucocorticoid receptors) on which corticosterone act to modulate gene transcription in the brain { ADDIN EN.CITE

<EndNote><Cite><Author>Pitman</Author><Year>1988</Year><RecNum>1534</RecNum><DisplayText>(51)</DisplayText><record><rec-number>1534</rec-number><foreign-keys><key app="EN" db-id="50wxdpzd9vd5r7e9t5b595djrfptrxw9avp">1534</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Pitman, David L</author><author>Ottenweller, John E</author><author>Natelson, Benjamin H</author></authors></contributors><titles><title>Plasma corticosterone levels during repeated presentation of two intensities of restraint stress: chronic stress and habituation</title><secondary-title>Physiology & behavior</secondary-title></titles><periodical><full-title>Physiology and Behavior</full-title><abbr-1>Physiol. Behav.</abbr-1><abbr-2>Physiol Behav</abbr-2><abbr-3>Physiology &

Behavior</abbr-3></periodical><pages>47-

55</pages><volume>43</volume><number>1</number><dates><year>1988</year></dates>
><isbn>0031-9384</isbn><urls></urls></record></Cite></EndNote>}. High level of
circulating corticosterone may have also contributed to increased global DNA methylation in
the PFC through altering the expression DNMT1 in the offspring of WF and NF, and DNMT3a
in the offspring of WF leading to increased susceptibility to stress-induced anxiety. Several
studies associated high serum corticosterone level with altered histone acetylation, DNMT1
transcription and global DNA methylation in an animal models of stress-induced emotional
alterations { ADDIN EN.CITE { ADDIN EN.CITE.DATA }}.

Overall, these results suggest that chronic exposure to cannabinoids during adolescence may
lead to a transgenerational transfer of stress susceptibility to the offspring through a transfer of
epigenetic marks. These inherited traits can be triggered by increased levels of circulating
corticosterone and expressed as an anxiety-like behaviour. This proposal is corroborated by
the fact that the unpredicted stress regimen differently altered DNA methylation profile in the
PFC. It did specifically increase global DNA methylation in WF offspring in comparison to
NF offspring. Accordingly, Vialou and colleagues found that Increased global DNA
methylation within the cortico-limbic brain regions results in reduced expression of several
genes implicated in stress coping and resilience, such as glucocorticoid receptors, brain-derived
neurotrophic factor and corticotrophin release factor { ADDIN EN.CITE
<EndNote><Cite><Author>Vialou</Author><Year>2013</Year><RecNum>1537</RecNu
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name="Journal Article">17</ref-type><contributors><authors><author>Vialou,
Vincent</author><author>Feng, Jian</author><author>Robison, Alfred
J</author><author>Nestler, Eric
J</author></authors></contributors><titles><title>Epigenetic mechanisms of depression and
antidepressant action</title><secondary-title>Annual review of pharmacology and
toxicology</secondary-title></titles><periodical><full-title>Annual Review of
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1><abbr-2>Annu Rev Pharmacol Toxicol</abbr-2><abbr-3>Annual Review of Pharmacology
& Toxicology</abbr-3></periodical><pages>59-
87</pages><volume>53</volume><dates><year>2013</year></dates><isbn>0362-

1642</isbn><urls></urls></record></Cite></EndNote>}. Further corroborating the involvement of DNA methylation in the observed anxiety-like behaviour related to paternal exposure to WIN is the increased DNMT1 transcription level in WF offspring and increased DNMT3a transcription specifically in animals subjected to stress. It is also noteworthy that the stress regimen upregulated DNMT1 transcription in the offspring in an independent manner to paternal exposure to cannabinoids, and had no effect on DNMT3a transcription in the offspring of NF. These data highlight the complexity of the interplay between the paternal inheritance of susceptibility to stress and stress-induced DNA methylation changes. Both, the inheritance of epigenetic traits from WF and unpredicted stress may cause the nervous system to undergo molecular changes that influence its susceptibility or resilience to stress. Also, this data suggests a role for sex chromosomes and/or imprinted genes in carrying the epigenetic marks responsible for the behavioural alterations in the offspring. it was shown that chronic exposure to cannabinoids in adolescent female rats causes morphine sensitization in the offspring in the absence of in utero exposure { ADDIN EN.CITE <EndNote><Cite><Author>Vassoler</Author><Year>2013</Year><RecNum>1538</RecNum><DisplayText>(55)</DisplayText><record><rec-number>1538</rec-number><foreign-keys><key app="EN" db-id="50wxdpzd9vd5r7e9t5b595djrfptrxw9avp">1538</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Vassoler, Fair M</author><author>Johnson, Nicole L</author><author>Byrnes, Elizabeth M</author></authors></contributors><titles><title>Female adolescent exposure to cannabinoids causes transgenerational effects on morphine sensitization in female offspring in the absence of in utero exposure</title><secondary-title>Journal of psychopharmacology</secondary-title></titles><periodical><full-title>Journal of psychopharmacology</full-title></periodical><pages>1015-1022</pages><volume>27</volume><number>11</number><dates><year>2013</year></dates><isbn>0269-8811</isbn><urls></urls></record></Cite></EndNote>}. Also, cocaine and alcohol exposure were associated to dysregulated DNMT1 transcription in testes and sperm of adult male rodents, hence, presenting a high-risk factor for heritable epigenetic changes { ADDIN EN.CITE <EndNote><Cite><Author>He</Author><Year>2006</Year><RecNum>1539</RecNum><DisplayText>(56, 57)</DisplayText><record><rec-number>1539</rec-number><foreign-keys><key app="EN" db-id="50wxdpzd9vd5r7e9t5b595djrfptrxw9avp">1539</key></foreign-keys><ref-type

name="Journal Article">17</ref-type><contributors><authors><author>He, Fang</author><author>Lidow, Irina A</author><author>Lidow, Michael S</author></authors></contributors><titles><title>Consequences of paternal cocaine exposure in mice</title><secondary-title>Neurotoxicology and teratology</secondary-title></titles><periodical><full-title>Neurotoxicology and Teratology</full-title><abbr-1>Neurotoxicol. Teratol.</abbr-1><abbr-2>Neurotoxicol Teratol</abbr-2><abbr-3>Neurotoxicology & Teratology</abbr-3></periodical><pages>198-209</pages><volume>28</volume><number>2</number><dates><year>2006</year></dates><isbn>0892-0362</isbn><urls></urls></record></Cite><Cite><Author>Ouko</Author><Year>2009</Year><RecNum>1540</RecNum><record><rec-number>1540</rec-number><foreign-keys><key app="EN" db-id="50wxdpzd9vd5r7e9t5b595djrfpttrxw9avp">1540</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Ouko, Lillian A</author><author>Shantikumar, Katpaham</author><author>Knezovich, Jaysen</author><author>Haycock, Philip</author><author>Schnugh, Desmond J</author><author>Ramsay, Michele</author></authors></contributors><titles><title>Effect of alcohol consumption on CpG methylation in the differentially methylated regions of H19 and IG-DMR in male gametes—Implications for fetal alcohol spectrum disorders</title><secondary-title>Alcoholism: Clinical and Experimental Research</secondary-title></titles><periodical><full-title>Alcoholism: Clinical and Experimental Research</full-title></periodical><pages>1615-1627</pages><volume>33</volume><number>9</number><dates><year>2009</year></dates><isbn>0145-6008</isbn><urls></urls></record></Cite></EndNote>}. This suggests, that chronic cannabinoid exposure during adolescence could also cause direct epigenetic modifications in sperm and/or testes that could be carried to the offspring. Future studies are needed to determine whether the epigenetic and behavioural changes observed in the offspring of WF are related to a disruption of the endocannabinoid system and its interaction with other neuroendocrine systems in the fathers or direct effect on testes.

Overall, the present study provided evidence for possible paternal transmittance of stress sensitivity traits to the offspring following chronic cannabinoid exposure and highlighted a role for DNA methylation in stress-induced anxiety. Future studies are needed to determine whether

cannabinoids-related behavioural alterations are passed to subsequent generations F2 and F3, and also to examine the epigenome in paternal sperm to determine which specific epigenetic marks are responsible on stress vulnerability in the offspring.

References

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Figure legends:

Fig. 1: Schematic representation of the experimental design used to assess the behavioural effects of stress in the offspring of Win55,212-2 (1.2 mg/kg) exposed fathers.

Fig.2: Effect of chronic exposure to Win55,212-2 (1.2 mg/kg) during adolescence on locomotor activity in the offspring when subjected to one week of unpredicted stress. Results are presented as means \pm SEM, two-way ANOVA (n=8 per group).

Fig.3: Effect of chronic exposure to Win55,212-2 (1.2 mg/kg) during adolescence on anxiety-like behaviour in the offspring when subjected to one week of unpredicted stress. Results are presented as means \pm SEM, *p<0.001, Bonferroni post-hoc test (n=8 per group).

Fig.4: Effect of chronic exposure to Win55,212-2 (1.2 mg/kg) during adolescence on object recognition memory in the offspring when subjected to one week of unpredicted stress. Results are presented as means \pm SEM, two-way ANOVA (n=8 per group).

Fig.5: Effect of chronic exposure to Win55,212-2 (1.2 mg/kg) during adolescence on plasmatic corticosterone levels in WF and NF offspring when subjected to unpredicted stress. Results are presented as means \pm SEM. Two-way ANOVA (n=8 per group).

Fig.6: Effect of chronic exposure to Win55,212-2 (1.2 mg/kg) during adolescence on global DNA methylation in the PFC of WF and NF offspring when subjected to one week of unpredicted stress. Results are presented as means \pm SEM, *p<0.01, Bonferroni *post-hoc* test (n=8 per group).

Fig.7: Effect of chronic exposure to Win55,212-2 (1.2 mg/kg) during adolescence on DNMT1 (A) and DNMT3a (B) mRNA expression in the PFC of the offspring when subjected to one

week of unpredicted stress. Results are presented as means \pm SEM, * $p < 0.01$, Bonferroni *post-hoc* test (n=8 per group).

Figures:

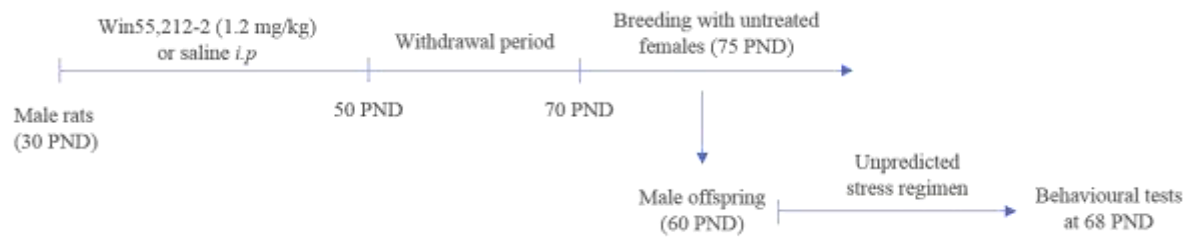


Fig.1: Schematic representation of the experimental design used to assess the behavioural effects of stress in the offspring of Win55,212-2 (1.2 mg/kg) exposed fathers.

PND: Post-natal day

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Fig.2: Effect of chronic exposure to Win55,212-2 (1.2 mg/kg) during adolescence on locomotor activity in the offspring when subjected to one week of unpredicted stress. Results are presented as means \pm SEM, two-way ANOVA (n=8 per group).

NF: offspring of non-exposed fathers

WF: offspring of Win55,212-2 exposed fathers

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Fig.3: Effect of chronic exposure to Win55,212-2 (1.2 mg/kg) during adolescence on anxiety-like behaviour in the offspring when subjected to one week of unpredicted stress. Results are presented as means \pm SEM, * $p < 0.001$, Bonferroni *post-hoc* test (n=8 per group).

NF: offspring of non-exposed fathers

WF: offspring of Win55,212-2 exposed fathers

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Fig.4: Effect of chronic exposure to Win55,212-2 (1.2 mg/kg) during adolescence on object recognition memory in the offspring when subjected to one week of unpredicted stress. Results are presented as means \pm SEM, two-way ANOVA (n=8 per group).

NF: offspring of non-exposed fathers

WF: offspring of Win55,212-2 exposed fathers

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Fig.5: Effect of chronic exposure to Win55,212-2 (1.2 mg/kg) during adolescence on plasmatic corticosterone levels in WF and NF offspring when subjected to unpredicted stress. Results are presented as means \pm SEM. Two-way ANOVA (n=8 per group).

NF: offspring of non-exposed fathers

WF: offspring of Win55,212-2 exposed fathers

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Fig.6: Effect of chronic exposure to Win55,212-2 (1.2 mg/kg) during adolescence on global DNA methylation in the PFC of WF and NF offspring when subjected to one week of unpredicted stress. Results are presented as means \pm SEM, *p<0.01, Bonferroni *post-hoc* test (n=8 per group).

NF: offspring of non-exposed fathers

WF: offspring of Win55,212-2 exposed fathers

{ EMBED Prism5.Document }

Fig.7: Effect of chronic exposure to Win55,212-2 (1.2 mg/kg) during adolescence on DNMT1 (A) and DNMT3a (B) mRNA expression in the PFC of the offspring when subjected to one week of unpredicted stress. Results are presented as means \pm SEM, *p<0.01, Bonferroni *post-hoc* test (n=8 per group).

NF: offspring of non-exposed fathers

WF: offspring of Win55,212-2 exposed fathers